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Timing of gibberellin $A_{4/7}$ application for cone production in potted black spruce grafts

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Potted 5-year-old grafts of black spruce (*Picea mariana* (Mill.) B.S.P.) growing in either a heated greenhouse or an outdoor holding area were sprayed weekly at 200 $mg \cdot L^{-1}$ gibberellin $A_{4/7}$ for various durations and timings. The application began 1 to 6 weeks after vegetative bud break and continued until the end of lateral shoot elongation. Sprayings ended at the same time for all treatments, about 1 week before leaf primordial differentiation on the shoot apices. The best response in seed-cone production occurred when application began 2 weeks after bud break (midstage of rapid shoot elongation) and continued for 5 weeks; treatments were also effective when applications began 3 weeks after bud break or earlier and continued for 4 to 6 weeks. Treatments initiated later (4 to 6 weeks after bud break) were not effective. Grafts kept outdoors produced more seed cones than those kept indoors. The effects on cone production of gibberellin $A_{4/7}$ application at four different concentrations were compared by spraying for 6 weeks, beginning 1 week after vegetative bud burst. Gibberellin $A_{4/7}$ at 200 $mg \cdot L^{-1}$ was the lowest foliar spray concentration found to be effective in promoting seed-cone production.

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Des greffes d'épinette noire (*Picea mariana* (Mill.) B.S.P.) âgées de 5 ans et cultivées en pot dans une serre chauffée ou entreposées à l'extérieur furent arrosées sur une base hebdomadaire avec 200 $mg \cdot L^{-1}$ de gibbérelline $A_{4/7}$ à des moments et pendant des durées variables. L'application a débuté 1 à 6 semaines après l'éclosion des bourgeons à feuilles et s'est poursuivie jusqu'à la fin de la période d'élongation des pousses latérales. Les arrosages ont pris fin au même moment pour tous les traitements; soit environ 1 semaine avant la différenciation du primordium foliaire à l'apex des pousses. La meilleure réponse en terme de production de cônes femelles a été obtenue lorsque les arrosages débutaient 2 semaines après l'éclosion des bourgeons (stade intermédiaire de l'élongation rapide de la pousse) et continuaient pendant 5 semaines; les traitements étaient aussi efficaces lorsque les arrosages commençaient 3 semaines après l'éclosion des bourgeons ou avant et continuaient pendant 4 à 6 semaines. Les traitements commencés plus tard (4 à 6 semaines après l'éclosion des bourgeons) n'étaient pas efficaces. Les greffes gardées à l'extérieur ont produit plus de cônes femelles que celles qui étaient à l'intérieur. Les effets de quatre concentrations différentes de gibbérelline $A_{4/7}$ sur la production de cônes ont été comparés avec une application hebdomadaire pendant 6 semaines débutant 1 semaine après l'éclosion des bourgeons à feuilles. La gibbérelline $A_{4/7}$ à 200 $mg \cdot L^{-1}$ était la plus faible concentration capable de promouvoir la production de cônes femelles.

[Traduit par la rédaction]

Introduction

The promotive effect of gibberellin $A_{4/7}$ ($GA_{4/7}$) application on cone-bud production has been well documented in Pinaceae species (Pharis *et al.* 1987). The effectiveness is often enhanced when applied in conjunction with heat treatment, root pruning, or water stress. This technology has offered tree breeders earlier and more abundant pollen and seed cones on newly established grafts and young seedlings for breeding to procure seed for progeny testing, and it has the potential to shorten the breeding cycle for advanced-generation breeding.

Timing of $GA_{4/7}$ application for promotion of cone production has been defined for some spruce (*Picea*) species (Marquard and Hanover 1984; Ross 1985; Ho 1989). Marquard and Hanover (1984) reported that $GA_{4/7}$ application should be initiated before meristematic differentiation, which occurs soon after shoot elongation. Ross (1985) reported that the effective period appeared to include the entire duration of shoot elongation. Ho (1991) found that treatment initiated about the time of leaf primordial differentiation was not effective. However, the optimal dura-

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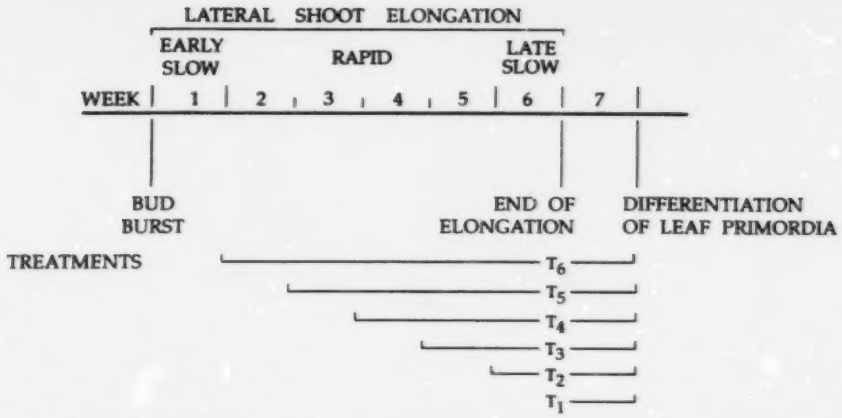

FIG. 1. Seasonal pattern of lateral shoot elongation in black spruce and the treatments of foliar gibberellin $A_{4/7}$ spraying. T_i, treatment; where i indicates the number of weekly sprayings.

TABLE 1. Average number of seed and pollen cones per graft (±SE) and percentage of grafts producing cones on potted black spruce grafts that were growing indoors or outdoors, and sprayed weekly with 200 mg·L^{-1} $GA_{4/7}$ for a 6- (T_6), 5- (T_5), 4- (T_4), 3- (T_3), 2- (T_2), and 1-week (T_1) period beginning from 1 to 6 weeks after vegetative bud burst

No. of weeks after bud burst (treatment)	Avg. no. of seed cones*		% of trees producing seed cones		Avg. no. of pollen cones*		% of trees producing pollen cones	
	Indoors	Outdoors	Indoors	Outdoors	Indoors	Outdoors	Indoors	Outdoors
6 (T_1)	2.8±1.1*a*	24.8±7.4*a*	50.0	65.0	0.9±0.7*a*	26.5±18.0*a*	11.1	20.0
5 (T_2)	7.0±2.4*a*	52.3±11.7*ab*	72.2	80.0	1.6±1.4*a*	0.1±0.1*a*	16.7	5.0
4 (T_3)	24.1±5.9*ab*	64.3±15.4*ab*	90.0	89.5	0.4±0.3*a*	6.5±4.7*a*	10.0	10.5
3 (T_4)	62.0±12.2*b*	107.4±13.8*b*	84.2	100	0.5±0.3*a*	7.3±6.6*a*	15.8	26.3
2 (T_5)	78.6±15.7*b*	119.4±21.9*b*	95.0	100	1.1±0.6*a*	0*a*	20.0	0
1 (T_6)	69.8±16.4*b*	111.1±22.4*b*	88.2	100	1.1±0.1*a*	0.5±0.4*a*	5.9	15.8

*Means within a column followed by the same letter do not differ significantly at the 0.05 level based on Scheffe's test (Steel and Torrie 1980).

TABLE 2. Analysis of variance of seed-cone production (square-root transformation) among the treatments listed in Table 1

Source	df	MS	*F*	*p*
Environments (E)	1	412.6393	53.07	0.0001
Treatments (T)	5	257.3224	33.09	0.0001
Clones (C)	9	67.8486	8.73	0.0001
E×T	5	4.6826	0.60	0.6983
E×C	9	31.8466	4.10	0.0002
T×C	45	13.6285	1.75	0.0097
E×T×C	45	11.6709	1.50	0.0457
Error	108	7.7755		

tion of weekly spraying is not well defined, although Pharis and Ross (1986) have recommended 4 to 6 weeks. In addition, $GA_{4/7}$ at 200 mg·L^{-1} has been the lowest concentration used for foliar spraying (Chalupka 1981; Hall 1988), so it is not known whether lower concentrations are effective. Using black spruce (*Picea mariana* (Mill.) B.S.P.) grafts, the present study was designed to define the optimal duration of $GA_{4/7}$ application for cone production, and to examine the effectiveness of its application at low concentrations to improve efficiency of cone production.

Material and methods

Black spruce was established in 4.5-L pots in 1983 by grafting scions from plus trees onto white spruce (*Picea glauca* (Moench) Voss) seedling rootstocks. The grafts were repotted into 15-L pots with a 5:4:1 (v/v/v) mixture of peat–perlite–sand in 1986. The experiments were carried out in 1988 when grafts averaged about 1 m in height.

During the various treatments, grafts were well watered and fertilized once a week with a 20:20:20 N–P–K fertilizer containing minor elements, while growing in either a heated greenhouse or an outdoor holding area. The greenhouse was maintained at 30°C during the day and 20°C at night, but the maximal daytime temperatures were often over 30°C, twice reaching 38°C for a short period of time around noon, because of the unusually hot, dry spring and summer in 1988.

Timing and duration of spraying

Twelve ramets for each of 10 clones were placed in the heated greenhouse from just before vegetative bud burst through the week after the last $GA_{4/7}$ spraying; another 12 ramets per clone were left outdoors.

Two ramets per clone in each environment (20 ramets per treatment per environment) were sprayed weekly for 6- (T_6), 5- (T_5), 4- (T_4), 3- (T_3), 2- (T_2), or 1-week (T_1) intervals, beginning 1, 2, 3, 4, 5, or 6 weeks after vegetative bud burst, respectively (Fig. 1). The last spraying in each treatment occurred about 1 week after shoot elongation, and before leaf primordial differentiation on the shoot apices.

Spraying of $GA_{4/7}$ was scheduled based on the bud break, lateral shoot elongation, and the time of leaf primordial differentiation on the shoot apices. The spraying was not carried out beyond the period of shoot elongation because it has been shown to be ineffective after that period (Marquard and Hanover 1984; Ho 1989). Shoot elongation was divided into the stages of early slow, rapid, and late slow elongation (Ross 1985); the early slow shoot elongation lasted for about 1 week, the rapid shoot elongation for about 4 weeks, and the late slow shoot elongation for about 1 week,

TABLE 3. Average number of seed and pollen cones per graft (±SE) and percentage of grafts producing cones on potted black spruce grafts that were sprayed weekly with different concentrations of $GA_{4/7}$ for 6 weeks beginning 1 week after vegetative bud burst

$GA_{4/7}$ concn. ($mg \cdot L^{-1}$)	Avg. no. of seed cones*	% of trees producing seed cones	Avg. no. of pollen cones*	% of trees producing pollen cones
0	2.8 ± 1.0*a*	33.3	6.4 ± 2.8*a*	29.2
50	42.8 ± 9.2*b*	75.0	0.8 ± 0.4*a*	20.8
100	56.5 ± 9.5*bc*	87.5	5.6 ± 3.7*a*	16.7
200	81.3 ± 12.0*c*	95.8	0.6 ± 0.3*a*	16.7

*Means within a column followed by the same letter do not differ significantly at the 0.05 level based on Scheffe's test (Steel and Torrie 1980).

based on the weekly measurement of terminal shoots of the first-order lateral branches on ramets (one ramet per clone per treatment) growing both indoors and outdoors (Fig. 1). Foliage of all the grafts (both indoor and outdoor) was thoroughly wetted with a $GA_{4/7}$ solution using a fine-mist sprayer. The solution contained 200 $mg \cdot L^{-1}$ $GA_{4/7}$, 15% aqueous ethanol, and 0.05% (v/v) Tween 20 surfactant. Seed cones and pollen cones were counted after emergence from the bud scales in the year following treatments.

$GA_{4/7}$ concentration

Twenty-four grafts growing in the greenhouse received six weekly sprayings of 0, 50, 100, or 200 $mg \cdot L^{-1}$ $GA_{4/7}$ in the greenhouse, beginning 1 week after vegetative bud burst (timing comparable to treatment T_6). Spray formulation, moving grafts in and out of the greenhouse, monitoring of shoot elongation (three grafts per concentration), and data collection were as described in the study on timing and duration of spraying.

Data analysis

Data on seed- and pollen-cone production were normalized by a square-root ($\sqrt{x + 0.5}$) transformation before using the linear models procedure of SAS Institute Inc. (1985).

Results

Gibberellin $A_{4/7}$ weekly spraying for 4 to 6 weeks, beginning 1 to 3 weeks after vegetative bud burst, significantly ($p \leq 0.05$) increased seed-cone production relative to other treatment periods (Table 1). Grafts growing outdoors produced significantly ($p \leq 0.0001$) more seed cones than those growing indoors; environment accounted for 51.1% of the total variation (Table 2). The treatments, clones, and all the interactions except that between environments and treatments were significant (Table 2). Eighty-four percent or more of grafts sprayed for 4 weeks or longer produced seed cones. Treatments did not affect pollen-cone production. From 0 to 26% of grafts produced pollen cones, with an average of 0 to 27 pollen cones per graft, depending on treatment.

Differences in seed-cone production were significant ($p \leq 0.0001$) at four different $GA_{4/7}$ concentrations. Grafts sprayed at 200 $mg \cdot L^{-1}$ $GA_{4/7}$ produced the highest number of seed cones, averaging 81 cones per graft, with 96% of the grafts producing cones (Table 3). The cone production on grafts sprayed at 50 $mg \cdot L^{-1}$ $GA_{4/7}$ did not differ significantly ($p \leq 0.05$) from that of grafts sprayed at 100 $mg \cdot L^{-1}$ $GA_{4/7}$, but both concentrations were significantly ($p \leq 0.05$) better than the controls. The treatments had no effect on pollen-cone production. From 17 to 29% of grafts produced pollen cones, with an average of 0.6 to 6 pollen cones per graft (Table 3).

Discussion

Timing of the $GA_{4/7}$ application has been defined and should be initiated during the period of shoot elongation when the bud scales are being formed on the shoot apices, and before the morphological differentiation when needle primordia, microsporophylls, or bracts begin to be initiated on the apices (Marquard and Hanover 1984; Ho 1988*a*, 1989). Initiation of the application during and after the differentiation was found to be ineffective (Ho 1989). Ross (1985) further established that the effective treatment period for $GA_{4/7}$ application included the entire duration of shoot elongation for Engelmann spruce (*Picea engelmannii* Parry). This is supported by the results in black spruce (Table 1). However, treatment could be initiated as late as the mid-stage of rapid shoot elongation for best results in seed-cone production. The application of $GA_{4/7}$ during the period of bud-scale initiation could have influenced the course of biochemical processes and resulted in early termination of bud-scale formation and initiation of microsporophylls or bracts in reproductive buds (Ho 1988*b*).

Duration of spraying can be optimized for maximal cone production when timing of foliar spraying is based on vegetative bud phenology. Four weekly $GA_{4/7}$ applications appeared to be optimal for black spruce when initiated in the midstage of rapid shoot elongation (Table 1). Pharis and Ross (1986) reported that the application for 4 to 6 weeks was about optimal for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). In spruces, most researchers employed the same duration of weekly spraying as for Douglas-fir (Marquard and Hanover 1984; Cecich 1985; Ho 1988*a*; Ross 1988), and some reported effective results from 1 to 10 weeks of spraying (Pharis *et al.* 1986; Hall 1988).

Spraying outdoors was more effective than spraying indoors (Table 1). This could be attributed to the hot, dry spring and summer in 1988; the often overheated greenhouse could have had an adverse effect on seed-cone production. Ross (1985) indicated that the maximal effect of heat treatment was the late stage of slow shoot elongation; the treatment was inhibitory at any other time. Black spruce grafts were placed in the greenhouse 1 week after bud burst, and the early placement might have caused the reduction in seed-cone production.

Gibberellin $A_{4/7}$ concentrations that have been used for foliar spraying vary substantially within a species and among species. The concentrations ranged from 200 $mg \cdot L^{-1}$ (Chalupka 1981) to 1200 $mg \cdot L^{-1}$ (Ho 1988*a*). In combination with GA_3 (300 $mg \cdot L^{-1}$), 100 $mg \cdot L^{-1}$ was the lowest concentration that stimulated cone production in Scots pine

(*Pinus sylvestris* L.) (Luukkanen and Johansson 1980). The most commonly used concentrations were between 200 and 400 $mg \cdot L^{-1}$ (Chalupka 1981; Marquard and Hanover 1984; Ross 1988), and both concentrations were found to be equally effective in promoting seed cones in black spruce (Ho 1989). The present study found that a $GA_{4/7}$ concentration as low as 50 $mg \cdot L^{-1}$ could promote seed-cone production. However, neither 50 $mg \cdot L^{-1}$ nor 100 $mg \cdot L^{-1}$ was as effective as 200 $mg \cdot L^{-1}$. Thus, based on the present results and the reports by Hall (1988) and Ho (1989), $GA_{4/7}$ at 200 $mg \cdot L^{-1}$ appeared to be the optimal concentration for effectively promoting cone crops in black spruce (Table 3).

Gibberellin $A_{4/7}$ spraying did not promote pollen-cone production in black spruce (Tables 1, 3), and similar results were noted by Ho (1989) for black spruce and by Marquard and Hanover (1985) for white spruce. However, Hall (1988) reported that $GA_{4/7}$ spraying promoted production of pollen cones in black spruce and was more effective for pollen cones than for seed cones when carried out in early July. Other spruces were found to respond to the spraying by increasing production equally of both pollen and seed cones (Cecich 1985; Ross 1985, 1988).

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